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Fitness correlates of male coloration in a Lake Victoria cichlid fish

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Sexual selection by female choice has contributed to the rapid evolution of phenotypic diversity in the cichlid fish species flocks of East Africa. Yet, very little is known about the ecological mechanisms that drive the evolution of female mating preferences. We studied fitness correlates of male nuptial coloration in a member of a diverse Lake Victoria cichlid lineage, *Pundamilia nyererei*. In this species, male red coloration is subject to intraspecific sexual selection by female mate choice. Male nuptial coloration plays a critical role also in reproductive isolation between this species and the closely related sympatric species *P. pundamilia*. Here, we show that *P. nyererei* male coloration is carotenoid based, illustrating the potential for honest signaling of individual quality. In a wild population, we found that variation in male coloration was not associated with variation in a set of strongly intercorrelated indicators of male dominance: male size, territory size, and territory location. Instead, the 2 male characters that predominantly determine female choice, territory size and red coloration, may be independent predictors of male quality: males with bright red coloration and large territories had lower parasite infestation rates. As a result, female preferences tended to select against heavily parasitized males. Consistent with parasite-mediated sexual selection, males had higher and more variable parasite loads than females. *Key words:* carotenoid display, cichlid fish, Lake Victoria, parasite-mediated sexual selection, *Pundamilia nyererei*, speciation. *[Behav Ecol 17:691–699 (2006)]*

INTRODUCTION

The several hundred species of haplochromine cichlid fish endemic to Lakes Victoria and Malawi are textbook examples of rapid adaptive radiation (Fryer and Iles 1972; Schluter 2000; Kocher 2004). Female mate choice has played an important role in the phenotypic diversification of these species flocks: female preferences for male coloration exert sexual selection within species (Seehausen et al. 1999; Maan et al. 2004; Pauers et al. 2004) and maintain reproductive isolation between sympatric incipient and sibling species (Seehausen et al. 1997; Knight et al. 1998; Van Oppen et al. 1998; Seehausen 2000). Little is known, however, about the ecological mechanisms that determine the origin and evolution of these mating preferences: what do females gain by being choosy with regard to male coloration? Identifying the mechanisms by which female preferences evolve is fundamental for understanding mechanisms of rapid speciation.

Theoretical models that investigate the odds of speciation by sexual selection often rely on the assumption that female preferences are selectively neutral (or entail only a small cost) and do not enhance offspring survival (e.g., Lande 1981; Turner and Burrows 1995; Van Doorn et al. 2004; for reviews, see Panhuis et al. [2001] and Turelli et al. [2001]). This assumption allows evolution of preference and trait in many directions and facilitates divergence. However, numerous studies indicate that sexually selected traits reliably indicate individual quality (for reviews, see Andersson 1994; Candolin 2003; Neff and Pitcher 2005). Female preferences for males that provide genetic quality or direct benefits are subject to natural selection and may not diverge so easily (Kirkpatrick and Nuismer 2004; but see Lorch et al. 2003; Edelaar et al. 2004; Reinhold 2004). Therefore, to understand the role of sexual selection in speciation, we must identify the selection pressures acting on mating preferences.

We have previously demonstrated directional sexual selection on male red coloration in the Lake Victoria cichlid fish *Pundamilia nyererei* (Maan et al. 2004). Male coloration is important also in interspecific female mate choice, which is the main source of reproductive isolation between *P. nyererei* and its close relative *Pundamilia pundamilia* (Seehausen and Van Alphen 1998). Common garden and quantitative genetics experiments with *P. nyererei* indicate that both male coloration and female preferences are heritable (Seehausen et al. 1997; Haesler and Seehausen 2005), making a coevolutionary process feasible. Here, we study the other extreme of the "sexual selection continuum" (Kokko et al. 2002), where female selectivity may increase offspring health and survival. We investigate possible sources of selection on female mating preferences within *P. nyererei*. Lake Victoria haplochromine cichlids are female mouthbrooders, and males usually do not contribute to brood care. Direct benefits are therefore unlikely to influence female choice, but bright colors may signal heritable quality through a variety of mechanisms (Andersson 1994). We ask whether male coloration is an indicator of male quality and thereby a predictor of offspring viability. Specifically, we study the potential for parasite-mediated sexual selection (Hamilton and Zuk 1982) in a wild *P. nyererei* population.

Red, orange, and yellow ornaments are often due to carotenoid deposition (e.g., in fish: guppies [Kodric-Brown 1989], sticklebacks [Brush and Reisman 1965], and cichlids [Evans and Norris 1996]). Besides their role in color signals,

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carotenoids are attributed various beneficial physiological functions, particularly in immune defense (Olson and Owens 1998; Hill 1999). Animals obtain carotenoids from food (Goodwin 1984), but they are considered a scarce resource (Grether et al. 1999) and may constitute a trade-off between sexual ornamentation and survival (Folstad and Karter 1992; Lozano 1994). Carotenoid-based sexual ornaments thus fulfill the requirements of an honest signal of quality (Zahavi 1975), and several examples of this mechanism have been found, especially in birds (Saino et al. 1999; McGraw and Ardia 2003; Saks et al. 2003) and fish (Barber et al. 2001; Grether et al. 2004).

Here we test 1) whether the red and yellow colors of male *P. nyererei* are carotenoid based. Subsequently, we investigate in wild territorial males 2) whether yellow and red coloration and/or other visible characteristics predict parasite load and 3) whether the latter is related to variation in mating success measured in the wild. Because females may select high-quality mates by choosing males that are dominant in male–male competition (Cox and LeBoeuf 1977; Berglund et al. 1996), we also investigate the relationships between mating success, male coloration, parasite load, and indicators of male dominance such as male size, territory size, and territory location.

METHODS

Behavioral observations and fish collection

We studied the *P. nyererei* population at Makobe Island in the western Speke Gulf, Tanzania (Seehausen and Bouton 1997), between December 2000 and February 2001 (subsequently referred to as "2001") and between November 2002 and March 2003 ("2003"). The relatively clear water at this island allows underwater observations (Secchi reading mean ± standard error (SE) = 221 ± 7 cm in the study period [84 measurements]). *Pundamilia nyererei* is mainly zooplanktivorous and also feeds on insect larvae and Nostoc (Bouton et al. 1997). Mature *P. nyererei* males defend territories on the rocky bottom at 4–7 m water depth, and they attract females by vigorous courtship displays (Seehausen and Van Alphen 1998). After mating, the female leaves the territory carrying the fertilized eggs in her mouth. Females mouthbrood their clutches for about 3 weeks, and after first releasing them, guard the fry for another week (Seehausen 1996). Females and nonterritorial males inhabit the same depth range and are typically seen in large feeding shoals mixed with individuals of other zooplanktivorous species.

Observations on individually marked territorial males

Using SCUBA, we identified 28 territorial males in the year 2001 and collected between 5 and 10 observations of 10 min each for every male, yielding a total of 50–100 min observation time per male. Males were not observed more than once in a day. We recognized males individually by their coloration pattern and/or injuries or scars. Observations were carried out between 9:00 AM and 1:00 PM, and all observations for any one male were completed within a period of 6–32 days. We recorded all interactions between these males and conspecific females. Female response was defined as the number of courted females responding positively (i.e., approaching the male) to male courtship. We used the number of male quivering bouts displayed to a female as a measure of male courtship intensity. We measured water depth at the territory (T depth, meters) and territory size (T size, square meters); territorial borders were deduced from the positioning of the territory owner during aggressive behavior toward other fish. We counted and measured (diameter, centimeters) all rocks in each territory and determined the number of crevices between rocks that were used by the territory owner. Territories were bimodally distributed over the sampled depth range with concentrations at depths of 4.21 ± 0.06 m ($n = 11$) and 6.26 ± 0.06 m ($n = 17$).

In the year 2003, we found *P. nyererei* territories in a continuous depth range from 4.3 to 6.5 m in the same area of Makobe Island. Territory size and depth distribution did not differ between years (average territory size and depth: $t < 1.2$, $P > 0.24$; depth distribution: $\chi^2 = 5.67$, degrees of freedom [df] = 6, $P > 0.2$). We located 23 territorial males and measured the territories of 14 of these males. We caught 8 males holding measured territories and 9 males holding territories that were not measured. Behavioral observations were not conducted.

In both years, territorial males were caught in gillnets or by hook and line, both using SCUBA. They were slowly brought to the surface in transparent plastic bags, which minimized the occurrence of stress-induced color changes.

Observations on additional males and females

We collected an additional sample for parasite load analysis in 2001: 26 males and 19 females were caught in gillnets (length × height: 30 × 1.5 m; stretched mesh sizes of 12.5, 16.5, and 18.5 mm) at approximately 6 m water depth. Nets were set for a maximum of 1 h to make sure that males did not lose color. Female abundance at different depths was determined in 2003. We set 12.5-mm gillnets at 3.5-, 4-, 5-, and 6-m depth in the study area and collected 104 females in 380 min. This sample emanated from experimental fishing for other species, and we did not preserve these fish. Therefore, these catches could only be used to determine the numbers of *P. nyererei* females at different depths but not their gonadal maturation stages. To determine the depth distribution of females in relation to gonadal maturation stages, we collected a second sample at 3.5-, 4-, 5-, 6-, and 6.5-m depth also in 2003 (138 females).

Preservation

Immediately after capture, males were photographed for color analysis. They were sacrificed on melting ice and measured (standard length [SL], to the nearest 0.1 mm). Fish were preserved in 4% formalin, the body cavity slit open ventrally to allow preservation of organs and internal parasites. After 1–4 weeks, they were transferred to 30% ethanol, at least 1 week later to 60%, and again at least 1 week later to the final solution of 70%. They were then weighed (W, to the nearest 0.1 g), and condition factor (CF) was calculated as $CF = 100 \times (W/SL^3)$ (Sutton et al. 2000).

Color analysis

For photography, males were placed in a perspex cuvette with water and gently squeezed against the front window of the cuvette with a gray PVC sheet in the back. For all pictures, we adjusted white balance in PhotoShop 6.0 (Adobe Systems Inc., San Jose, CA) using a white patch (Kodak color card) attached to the front of the cuvette. We calculated color scores in SigmaScan Pro 4.0 (SPSS Inc., Chicago, IL). Following Maan et al. (2004), we used criteria delimiting the body area (excluding fins and eyes) covered by red and yellow based on a combination of hue and saturation (red: hue = 0–26 plus 232–255, saturation 40–97%; yellow: hue = 27–45, saturation 40–97%) and subsequently calculated the area of the fish body that matched these criteria. This yielded a percentage of body coverage for each color, subsequently referred to as "red score" and "yellow score". We similarly defined criteria for blackness to quantify the body coverage of the black vertical bars and ventral aspects of the body (intensity = 0–75; "black score").

In 2001, we used an SLR camera and 2 flashes on either side and subsequently digitized pictures. In 2003, we used a digital camera without flash. To enable comparison of color scores between years, we calculated color scores of the Kodak color card that was photographed with both the SLR and the digital camera ($n = 10$ pictures for each camera). This yielded a calibration factor of 1.16 for both red and yellow scores. Black score was too different between methods to allow for calibration. We indicate in the text where we use calibrated color scores.

Determination of parasite load and sexual maturity

With a dissecting microscope, we examined the skin, fins, gills, abdominal cavity, gonads, liver, and gastrointestinal tract and counted all parasites. Parasite identification followed Paperna (1996). We report parasite counts for each parasite species separately. We calculated additional summary variables as estimates of overall parasite infestation rate: total parasite load (TPL) is the sum of all parasites infecting one fish and PS is the total number of parasite species infecting one fish. For territorial males, we calculated a third summary variable (medium parasite load, MPL) that takes the differences in abundance between parasite species into account: for each species, we normalized the number of individuals infecting one fish with the medium parasite load of that species in the sample of territorial males of either year and summed these relative loads.

Sexually mature, gravid, females were those that were ready to spawn within a few days, that is, their ovaries were full with eggs that had a diameter of at least 80% of the maximum observed. Sexually mature males had their testes swollen to more than 80% of the maximum observed.

Skin pigment analysis

One female and one male, adult F1 offspring of wild-caught *P. nyererei* from Makobe, were sacrificed on ice. Of each fish, 2 skin samples of approximately 2 cm^2 were taken: one from the dorsum (red in males) and the other from the side of the fish (yellow in males; both brownish in females). These were dried for a few minutes and then weighed (g) and measured (mm^2). Pigments were extracted in acetone for 24 h. Absorption spectra of the acetone extracts, measured in the range of 220–600 nm in a spectrophotometer (Unicam UV1), showed that male skin samples significantly absorbed in the 420- to 470-nm range, whereas female skin samples did not. Acetone extracts of the male skin samples were evaporated overnight, redissolved in hexane, and absorption spectra determined. Carotenoid content of these extracts was estimated from absorbance at λ_{max} using the absorption coefficient $A^{1\%}_{1cm} = 2500$ for carotenoid mixtures (Britton et al. 1995). Besides carotenoids, orange and red color patterns in fish can contain drosopterins (Fox and Vevers 1960; Hudon et al. 2003). To detect these, hexane extracts were dried and redissolved in 30% EtOH, acidified with HCl to pH 2, and tumbled for 24 h at room temperature. For comparison, drosopterins were extracted from 50 *Drosophila melanogaster* heads using the same procedure. Extracts were analyzed using spectrophotometry and high-performance liquid chromatography (HPLC) (Waters 990 photodiode array) with an Allsphere ODS 2 column (5 μm) (15 cm × 4.6 mm) (Alltech) and a mobile phase of 70:20:10 (volume %) acetonitril:CH_2Cl_2:methanol (flow rate 1 ml/min, pressure 500 psi).

Data analysis

Comparisons of groups and bivariate relationships were analyzed using paired *t*-tests and Pearson correlations for normally distributed data and Mann–Whitney *U* tests, Wilcoxon signed-ranks tests, chi-square tests, and Spearman correlations for non–normally distributed data (SPSS 10.0 [SPSS Inc.]). Means of normally distributed data are reported with SEs. Multivariate relationships were analyzed with generalized linear models (GLMs) using R (Ihaka and Gentleman 1996; http://www.r-project.org). Models with counts as dependent variables used Poisson distributions and logarithmic link functions. The proportion of gravid females was analyzed with a binomial model and a logit link function. All other models assumed normal distributions; distributions of residuals were consistent with this assumption. Stepwise removal of nonsignificant variables from saturated models yielded minimal adequate models; significance was determined by *F*-tests examining the change in deviance after the removal of each variable. In Poisson models, test statistics were adjusted for over- and underdispersion (Venables and Ripley 2002).

To determine whether and how variation in parasite load was reflected in variation in characteristics of territorial males that could be assessed by females, we calculated minimal adequate GLMs with parasite load as dependent variable and the following male characteristics as independent variables: red score, yellow score, black score, SL, courtship intensity, and territory size and water depth. Because male red score, courtship intensity, and territory size determine female choice (Maan et al. 2004), we report GLM results for these traits separately. Courtship intensity data were collected only in 2001. In all other models, we pooled the data collected in 2001 and 2003 and included "year" as a factor. Because territory size varied with water depth, depth was included as a covariate in models with territory size as independent variable. For all tests of relationships with parasites, we applied sequential Bonferroni corrections (Sokal and Rohlf 1995), using $k =$ the number of parasite species analyzed or $k =$ the number of summary variables.

RESULTS

Yellow and red skin pigments are carotenoid based

The absorption spectrum of the hexane extract from the red skin sample showed a typical carotenoid pattern: a shoulder at 418 nm, one peak at 439 nm, and the highest peak at 468 nm. Total carotenoid content was 0.58 mg/g and 0.24 μg/mm^2. The yellow sample showed a very similar absorption spectrum: one peak at 416 nm, the highest peak at 439 nm, and a third peak at 467 nm. Carotenoid content was 0.16 mg/g and 0.085 μg/mm^2. Thus, the red skin sample contained approximately 3 times more carotenoid than the yellow skin sample. The EtOH extract of the yellow skin sample did not contain drosopterins. Both the red skin extract and the *Drosophila* extract showed a broad absorption peak at 476 nm, corresponding to the known absorption peak of drosopterins in acid solution (475 nm, Needham 1974). The retention times of this compound in the HPLC were identical for the fish and the *Drosophila* extracts (2.06 min). For the red fish skin sample, the contribution of drosopterins to the total absorption of visible light was small: peak absorbance amounted to only 3.5% of carotenoid peak absorbance.

Territorial males: male color is not related to body size or territory size

Male coloration did not covary with male size: none of the color scores were related to male size in either year (Pearson correlations, SL: all $P > 0.17$; weight: all $P > 0.29$). Likewise, male coloration was not related to territory size (Pearson correlations, all $P > 0.40$).

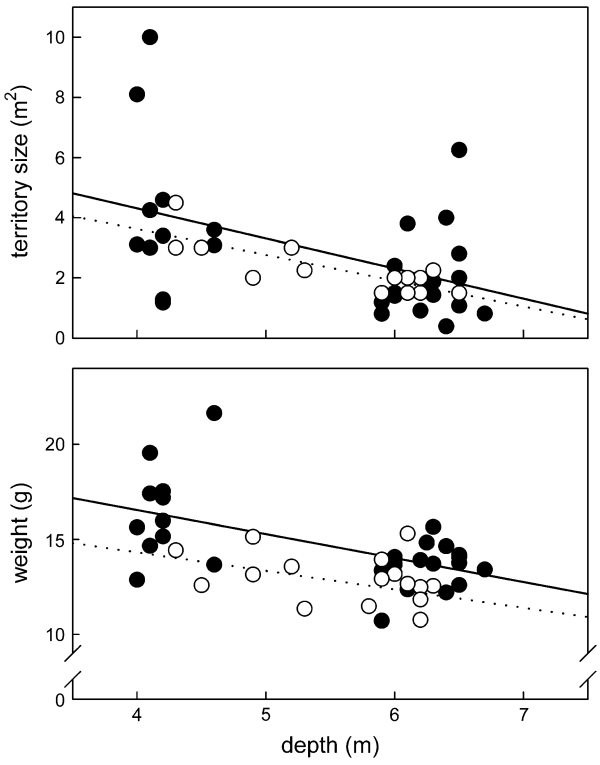

Figure 1
Territory size and male weight as functions of water depth in 2001 (filled circles, solid line) and 2003 (open circles, dotted line).

Both territory size and male size varied with water depth (Figure 1 and Table 1). Male territories were larger in shallow water in both years. In 2001, males in shallow water were larger and had higher CFs than males in deeper water. In 2003, there were nonsignificant trends in the same direction (Table 1). In 2001, males in deeper water had higher yellow scores but not red scores, and similar trends were present in 2003 (Table 1). Black scores tended to decrease with depth. The relationships between male size, territory size, and territory depth were not affected by male coloration (GLM; all $P > 0.20$ in both years). Thus, males with high (or low) color scores were not larger and did not hold larger territories in either year, but males in deep water were smaller and tended to have higher color scores.

Deep and shallow habitats differed in several ways. First, although the number of rocks in a territory was identical (median = 23 in both deep and shallow territories), rocks in shallow water were larger (rock diameter in shallow territories: 51 ± 4.6 cm [$n = 11$], deep: 34 ± 2.8 cm ($n = 17$), $t = 3.35$, $P = 0.0026$) and provided more interstitial crevices (median and range 4 [1–5] vs. 2 [1–4] per territory, Mann-Whitney U $Z = -2.057$, $P = 0.047$; data from 2001). Second, the total abundance (catch per effort) of adult females increased significantly with increasing depth (GLM estimate = 0.50 ± 0.095, $F_{1,2} = 28.5$, $P = 0.033$). As the proportion of gravid females (i.e., ready to spawn) was larger in shallower water, however (GLM estimate = −0.41 ± 0.061, $F_{1,3} = 46.6$, $P = 0.0064$; data from 2003), the resulting expected number of gravid females that males would encounter did not differ between depth classes ($\chi^2 = 3.32$, df = 3, $P = 0.34$). In line with this, depth did not influence female mate choice (Maan et al. 2004), and deep and shallow males did neither differ in the female response rates that they obtained ($n_1 = 17$, $n_2 = 11$, MWU $Z = -1.03$, $P = 0.31$) nor in the total number of interactions with females ($Z = -0.59$, $P = 0.58$) or the number of aggressive interactions with females ($Z = -1.16$, $P = 0.26$).

Territorial males caught in 2003 were significantly smaller than those caught in 2001 ($n_1 = 28$, $n_2 = 17$; SL: 80.9 ± 0.5 vs. 76.4 ± 0.7 mm, $t = 5.1$, $P < 0.001$; weight: 13.7 ± 0.4 vs. 12.7 ± 0.4 g, $t = 3.12$, $P = 0.0032$), but there was no difference in CF ($t = 0.812$, $P = 0.37$). In 2001, male condition decreased with increasing red score ($n = 28$, $r = -0.44$, $P = 0.020$). In 2003, this relationship was absent ($n = 17$, $r = 0.14$, $P = 0.60$). GLM analysis showed that this relationship differed significantly between years ($F_{1,42} = 4.98$, $P = 0.031$). Yellow score and black score were not related to body condition in either year (Pearson correlations, all $P > 0.27$). Males in 2003 had significantly higher scores for all colors ($t > 4.3$, $P < 0.001$). For black score, this difference may have been entirely due to the different cameras used. The differences in red and yellow scores however remained significant after calibration (red—2001: 17.8 ± 1.5, 2003: 34.9 ± 1.5, $t = 7.48$, $P < 0.001$; yellow—2001: 1.7 ± 0.4, 2003: 4.9 ± 0.7, $t = 3.85$, $P = 0.001$).

Parasites

Parasite composition

We found 7 species of macroscopic parasites: 2 endoparasites and 5 ectoparasites. In the skin and fins, we found encysted metacercariae belonging to the trematod genus *Neascus* (Digenea). Three gill parasites were relatively common: 2 species of ectoparasitic copepods (*Lamproglena monodi* [Lernaeidae] and *Ergasilus lamellifer* [Ergasilidae]) and 1 monogenean (*Cichlidogyrus* sp. [Dactylogyridae]). Encapsulated larvae of an

Table 1
Male size, condition, territory size, and color scores in 2001 and 2003: means, SEs, and test results for the differences between deep and shallow territorial males

	2001				2003			
	Shallow (11)	Deep (17)	t	P	Shallow (6)	Deep (11)	r	P
SL (mm)	82.6 ± 0.8	79.8 ± 0.6	2.95	0.0067	76.3 ± 1.6	76.5 ± 0.7	−0.18	0.48
Weight (g)	16.5 ± 0.8	13.6 ± 0.3	3.58	0.0035	13.4 ± 0.5	12.3 ± 0.5	−0.39	0.12
Condition (g/mm^3)	2.9 ± 0.1	2.7 ± 0.1	2.33	0.028	3.0 ± 0.2	2.7 ± 0.1	−0.28	0.28
Territory size (m^2)	4.1 ± 0.8	2.0 ± 0.4	2.70	0.012	3.0 ± 0.4	1.8 ± 0.1 ($n = 8$)	−0.80	<0.001
Red score (%)	12.9 ± 1.8	17.0 ± 2.1	1.37	0.18	27.8 ± 2.2	31.4 ± 1.5	+0.46	0.067
Yellow score (%)	0.7 ± 0.2	2.0 ± 0.5	2.36	0.029	2.4 ± 0.5	5.2 ± 0.8	+0.43	0.089
Black score (%)	10.4 ± 3.3	3.7 ± 1.3	1.91	0.079	46.7 ± 9.0	38.9 ± 6.1	−0.24	0.35

For 2003, red and yellow scores are calibrated for the difference in photography method; black scores are not calibrated. The cutoff point to categorize "deep" and "shallow" territories is 5.4-m depth (i.e., the middle of the depth range of *Pundamilia nyererei* territories in both years).

Table 2
Parasite load in 2001 and 2003

a) Parasite load

	Territorial males 2001 ($n = 28$)		Territorial males 2003 ($n = 17$)		Unidentified males 2001 ($n = 27$)		Unidentified females 2001 ($n = 19$)		b) Males ($n = 55$) versus females ($n = 19$) (2001)			c) Territorial males 2001 ($n = 28$) versus 2003 ($n = 17$)		
	%	Median (range)	%	Median (range)	%	Median (range)	%	Median (range)	MWU Z	P	Sign	MWU Z	P	Sign
Neascus sp.	89.3	2 (0–9)	100	5 (1–19)	46.2	0 (0–20)	52.6	1 (0–3)	1.49	0.14	♂ > ♀	3.11	**0.0019**	2001<2003
Lamproglena monodi	89.3	5 (0–12)	100	7 (2–15)	84.6	2 (0–14)	84.2	3 (0–9)	1.77	0.077	♂ > ♀	2.71	**0.0067**	2001<2003
Ergasilus lamellifer	78.6	1 (0–9)	64.7	2 (0–5)	53.8	1 (0–10)	36.8	0 (0–2)	2.90	**0.0038**	♂ > ♀	0.91	0.37	
Mollusc larvae	14.3	0 (0–1)	47.1	0 (0–12)	7.7	0 (0–1)	10.5	0 (0–1)	0.43	0.67		2.49	0.013	2001<2003
Cichlidogyrus sp.	100	18.5 (4–52)	88.2	6 (0–12)	96.2	7 (0–41)	100	4 (1–11)	5.24	<**0.001**	♂ > ♀	4.78	<**0.001**	2001>2003
Trematodes	10.7	0 (0–5)	0	0	11.5	0 (0–3)	5.3	0 (0–1)	0.85	0.40		1.38	0.17	
Nematodes	96.4	5.5 (0–136)	88.2	4 (0–20)	80.8	2.5 (0–112)	84.2	5 (0–56)	1.02	0.31		0.68	0.50	
TPL		38 (19–166)		24 (11–52)		16 (4–124)		16 (6–70)	2.42	**0.016**	♂ > ♀	−2.59	**0.0096**	2001>2003
PS		5 (3–6)		5 (3–6)		4 (1–6)		4 (2–6)	2.24	**0.025**	♂ > ♀	−0.47	0.64	

a) Parasite infestation rates of territorial males, unidentified males, and unidentified females. % Denotes parasite prevalence: the proportion of infected individuals. Median is the median number of parasites of the given taxon per individual fish. b) Differences in parasite load between males and females in 2001. c) Differences in parasite load between territorial males in 2001 and 2003. Test results that remain significant after sequential Bonferroni correction are given in bold. TPL, total parasite load; PS, total number of parasite species.

unidentified bivalve mollusc were present in the gills of a small number of fish. Larval nematodes (*Contracaecum* sp.) were commonly found in liver and abdominal cavity; trematodes (Digenea) were found in the intestines of a few fish in 2001 only.

Males have more parasites and larger variance in infestation rates than females

Males carried more parasites than females (Table 2). However, male *P. nyererei* are larger than females, and numbers of several parasites were related to fish size (GLM; results not shown). In a GLM with parasite loads as dependent variables and fish sex and size (SL and weight) as independent variables, males still carried significantly more *E. lamellifer* and *Cichlidogyrus* than females ($F_{1,72} > 5.7$, $P < 0.020$). Because infestation rates of some parasite species were higher in territorial males than in unidentified males (Table 2a), we also analyzed a subsample including only sexually mature fish (40 males, 10 females). This yielded similar results to the previous GLM analysis, except for a now significantly higher number of parasite species (PS) in males ($F_{1,48} = 9.28$, $P = 0.0038$). The variance in parasite infestation rates was also larger in males (Wilcoxon signed-ranks test comparing parasite load variance for each parasite species between males and females: $Z = -2.197$, df = 6, $P = 0.028$; including only sexually mature fish: $Z = -2.366$, df = 6, $P = 0.018$).

In 2003, TPL of territorial males was significantly lower than in 2001, mainly due to lower infestation rates with *Cichlidogyrus* (Table 2). We found significantly more *Neascus* and *L. monodi* in 2003. Parasite load was not related to body condition in either year (males and females: all $F < 3.97$, $P > 0.07$). Including color scores in the models for males did not influence these results.

Red score and territory size predict male parasite load

Of the different parasite species, only the nematodes were significantly related to male characteristics that could be assessed by visiting females: high red score and small size (SL) predicted low nematode load ($F \geq 7.78$, $P \leq 0.008$; Table 3a). Large fish (SL) tended to carry fewer *E. lamellifer* ($P = 0.019$, Bonferroni-corrected critical $P = 0.010$) and mollusc larvae ($P = 0.022$, Bonferroni-corrected critical $P = 0.013$) in their gills. There were nonsignificant trends for negative relationships between territory size and *L. monodi* infection ($P = 0.084$) and between red score and *Cichlidogyrus* ($P = 0.082$). Intestinal trematodes were not analyzed separately because of their low incidence, but they were included in the summary variables.

Both male red score and territory size were significantly negatively related to TPL ($F \geq 8.32$, $P \leq 0.0070$), and a large territory also predicted low MPL ($F_{1,33} = 11.06$, $P = 0.0022$) (Figure 2). The number of parasite species infecting individual fish was not significantly related to any of the variables. Male courtship behavior was not significantly related to parasite load ($P > 0.026$; Bonferroni-corrected critical $P < 0.017$). Likewise, male yellow score and black score were not related ($P > 0.10$) to infestation rates with any of the parasite species.

Females tend to select against heavily parasitized males

Previous work showed that female response rate was positively related to male red score, courtship intensity, and territory size (Maan et al. 2004). There was a tendency for these preferences to yield selection against heavily parasitized males: there were negative relationships ($P < 0.05$) between female response rate and male parasite load for *Neascus* and *Cichlidogyrus* infection and for TPL (Table 3b). After Bonferroni correction, however, these relationships were no longer significant.

DISCUSSION

The red and yellow coloration of *P. nyererei* males is carotenoid based. Extracts from the red skin areas contained 3 times as much carotenoid as the yellow skin extracts. Drosopterins, present in the red skin extracts, contributed less than 4% to total absorbance. Because we used laboratory-bred individuals for the pigment analysis, we cannot rule out the possibility that the quality and quantity of the pigments encountered may differ from those in wild fish. However, the colors expressed by laboratory-bred *P. nyererei*, fed with a mixture of fresh shrimps and peas, are indistinguishable from those expressed by wild-caught individuals. Further, the differences in coloration that exist between different wild *P. nyererei* populations are maintained in common garden experiments in the laboratory (Seehausen et al. 1997).

We found no evidence that the redness of male coloration, which is the most important criterion for female mate choice (Maan et al. 2004), is important in male dominance. It was

Table 3
Parasite load and sexual selection

	a) Male parasite load and characteristics that can be assessed by visiting females													b) Male parasite load and female response (2001)	
	Minimal adequate model				Relation to red score			Relation to territory size			Relation to courtship intensity (2001)				
	Variable	Estimate ± SE	F	P	Estimate ± SE	$F_{1,42}$	P	Estimate ± SE	$F_{1,33}$	P	Estimate ± SE	$F_{1,26}$	P	r_s	P
Neascus sp.	SL	−0.043 ± 0.039	1.19	0.28	+0.002 ± 0.017	0.01	0.93	−0.017 ± 0.020	0.78	0.38	−0.183 ± 0.258	0.52	0.48	−0.43	0.023
Lamproglena monodi	T size	−0.024 ± 0.014	3.17	0.084	−0.010 ± 0.012	0.63	0.43	−0.024 ± 0.014	3.17	0.084	−0.190 ± 0.197	0.96	0.34	−0.13	0.52
Ergasilus lamellifer	SL	−0.124 ± 0.051	5.91	0.019	+0.012 ± 0.021	0.35	0.56	−0.037 ± 0.023	2.96	0.094	−0.464 ± 0.336	2.03	0.17	−0.01	0.97
Mollusc larvae	SL	−0.244 ± 0.104	5.68	0.022	−0.020 ± 0.057	0.12	0.73	−0.006 ± 0.074	0.01	0.93	+0.295 ± 0.682	0.17	0.68	0.20	0.31
Cichlidogyrus sp.	Red	−0.019 ± 0.011	3.17	0.082	−0.019 ± 0.011	3.17	0.082	−0.017 ± 0.010	2.74	0.11	−0.172 ± 0.177	0.98	0.33	−0.39	0.039
Trematodes														−0.19	0.32
Nematodes	Red	−0.094 ± 0.027	10.90	**0.0020**	−0.081 ± 0.030	7.78	**0.0079**	−0.086 ± 0.049	4.51	0.041	−0.518 ± 0.685	0.61	0.44	−0.26	0.18
	SL	+0.194 ± 0.065	8.77	**0.0051**											
TPL	Red	−0.024 ± 0.011	9.59	**0.0041**	−0.031 ± 0.010	8.83	**0.0049**	−0.034 ± 0.012	9.97	**0.0034**	−0.309 ± 0.203	2.43	0.13	−0.39	0.038
	T size	−0.028 ± 0.010	8.32	**0.0070**											
MPL	T size	−0.032 ± 0.011	9.22	**0.0047**	−0.020 ± 0.011	4.32	0.044	−0.036 ± 0.012	11.06	**0.0022**	−0.400 ± 0.175	5.55	0.026	−0.32	0.10
	Red	−0.014 ± 0.011	4.16	0.050											
PS	T size	−0.005 ± 0.003	3.17	0.084	−0.006 ± 0.004	2.08	0.16	−0.005 ± 0.003	3.17	0.084	−0.036 ± 0.051	0.51	0.48	−0.14	0.48

a) Relationships between male parasite load and male characteristics that can be assessed by visiting females. Except for the analyses involving courtship intensity, data of 2001 and 2003 are pooled, and "year" is included as a factor in the models. b) Relationships between male parasite load and female response in 2001. Test results that remain significant after sequential Bonferroni correction are given in bold. MPL, medium parasite load.

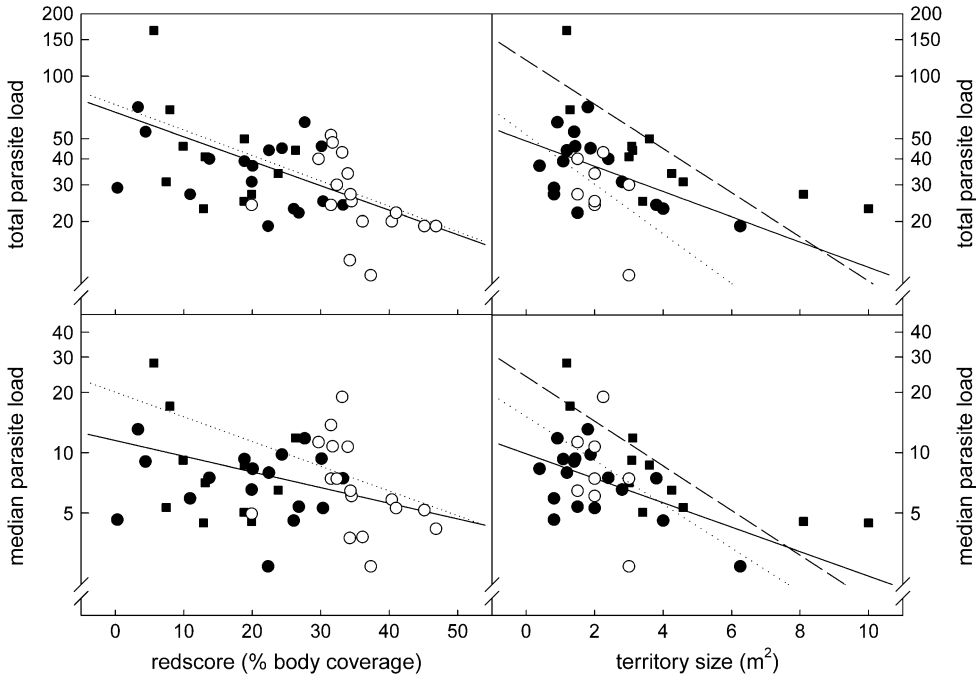

Figure 2
Male parasite load and characteristics that can be assessed by females. TPL is the sum of all parasites infecting one fish and MPL is the sum of normalized parasite loads. Filled symbols indicate territorial males of 2001 in deep (filled circles) and in shallow (filled squares) water and open circles indicate territorial males of 2003. Left panels: TPL and MPL in relation to red score. Solid lines represent 2001 males and dotted lines represent 2003 males. For 2001, red scores are adjusted for photography method (i.e., multiplied by 1.16; see Methods). The trend in the lower left panel is not significant (see Table 3; $P = 0.044$; Bonferroni-corrected critical $P = 0.025$). Right panels: TPL and MPL in relation to territory size. Solid lines represent males with territories in deep water (2001), dashed lines represent males with territories in shallow water (2001), and dotted lines represent 2003 males.

neither related to male body size nor to territory size. Territories in shallow water were larger, provided more shelter, and were occupied by larger males. Because body size predicts fighting ability in male *Pundamilia* (Dijkstra et al. 2005), the observed distribution of males may result from competition among males with shallow water being the preferred habitat. At Makobe Island, both sexes of *P. nyererei* are most abundant around 6-m depth (Seehausen and Bouton 1997; M. Maan, personal observation), suggesting that food conditions are not better in shallow water. Possibly, the larger rocks and greater number of crevices in shallow water provide better opportunities for predator avoidance. Moreover, territorial *P. nyererei* males compete for territories also with males of other haplochromine species. There is a general interspecific trend for larger species to inhabit shallower waters (Seehausen et al. 1998). In our study, males in shallow water had frequent aggressive interactions with *Neochromis omnicaeruleus* males (which are 30% larger), whereas males in deep water had disputes predominantly with *Pundamilia* "pink anal" males, which are similar in size to *P. nyererei* (Seehausen 1996).

Most fish show indeterminate growth. Irrespective of the mechanism behind the observed distribution of territorial males, male size may thus be a reliable indicator of survival, which in turn is expected to reflect genetic quality (Brooks and Kemp 2001). However, we did not find evidence that *P. nyererei* females preferred larger males or males with territories in shallower water. Assuming that female choice is adaptive, this suggests that these traits do not reflect male quality or that other visible cues are better indicators.

Indeed, our data suggest that if female mating preferences in *P. nyererei* are aimed at avoiding heavily parasitized males, females should pay attention to male red coloration and territory size. In 2 samples of territorial males that we took with an interval of 2 years, red coloration and territory size emerged as the best predictors of male parasite load. For most individual parasite species, there were trends for negative correlations with both characters. This corresponds with the observation that male red coloration and territory size are important criteria for female mate choice in this species (Maan et al. 2004). To our knowledge, this is only the second study of parasite-mediated sexual selection in cichlid fish (see also Taylor et al. 1998) and the first to implicate a carotenoid trade-off. Parasite load does not necessarily reflect the genetic quality of the immune system (Shykoff and Widmer 1996; Getty 2002): parasites may affect overall health and consequently weaken male competitive ability and color expression. However, we did not find any relationship between parasite load and body condition. Assuming that foraging efficiency and physiological trade-offs have a genetic basis, it seems unlikely that the observed variation in parasite load is due to environmental effects alone. Therefore, our results may rather suggest that female choice for males that are bright red and possess large territories has evolved under selection for heritable fitness.

The result that redness rather than yellowness is related to both female choice and parasite load is consistent with the idea that red is a more costly carotenoid display than yellow (Hill 1996) and is supported by the finding that the red areas of the male skin contain much higher carotenoid concentrations than the yellow areas.

The relationship between TPL and red coloration was similar in 2001 and 2003, but the territorial males in the 2003 sample were brighter red and carried fewer parasites, mainly due to lower infestation rates with *Cichlidogyrus* (Figure 2). We may speculate that female choice is driving the population temporarily toward lower parasite loads and associated

increases in red score, until another parasite species or another genotype of the same species comes to prevalence and causes a renewed increase in infestation rate. Perhaps more likely, infestation rates of *Cichlidogyrus* may have been lower in 2003 for environmental reasons and subsequently have allowed for higher average redness. Territorial males were smaller in 2003 than in 2001, but this difference seems to be unrelated to the difference in red score or parasite load: male size was not related to color, and parasites with higher infestation rates in larger fish (e.g., nematodes) were not less abundant in 2003.

Our observations that several parasite species occur in significantly larger numbers in males than in females, especially in territorial males (Table 2), and that infestation rates are significantly more variable in males, hint at male-specific trade-offs between immune defense and reproductive investment. These may be mediated by carotenoid-based male breeding coloration. Evidence for carotenoid limitation in males but not females has been found in guppies (Grether et al. 2004). However, the parasite species that was most strongly related to male coloration in our study, the nematode *Contracaecum*, did not differ in abundance between males and females. Conversely, the 2 species that were more abundant in males (*Ergasilus*, *Cichlidogyrus*) were not significantly related to male coloration. Perhaps the different parasite loads in males and females in *P. nyererei* are related to ecological differences between the sexes (Reimchen and Nosil 2001). Either way, the higher infestation rates of males make the production of carotenoid-based coloration particularly costly for males.

In contrast to selection for arbitrary traits, sexual selection for heritable fitness has been proposed to constrain rather than promote species divergence (Kirkpatrick and Nuismer 2004). This implies that our results present a challenge for the hypothesis that sexual selection has contributed to the divergence of *P. nyererei* and its close relative *P. pundamilia*. However, *P. nyererei* and *P. pundamilia* differ in diet and depth range (Seehausen 1996), which likely entails differences in parasite exposure. If heritable fitness in the face of different parasite communities is most honestly signaled by different color traits, divergent sexual selection for extreme male coloration (Maan et al. 2004; Pauers et al. 2004) may interact with divergent selection due to preferences for locally adapted males (Arnegard and Kondrashov 2004; Edelaar et al. 2004; Reinhold 2004). Likewise, selection for heritable fitness may interact with sensory drive if ambient light differs between shallow and deep water (Maan et al. 2006). Further work should evaluate the relative importance of these mechanisms for haplochromine speciation.

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